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Metabolic and Physical Control of Cell Elongation Rate

IN VIVO STUDIES IN *NITELLA*[1]

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ABSTRACT

Several levels of control of elongation rate are revealed through the detailed study of responses of the *Nitella* internode to abrupt shifts in turgor. The immediate response, which apparently reflects the physical state of the cell, is approximately described by the equation $r = (P - Y)m$ where r is rate, P is pressure, Y is the wall's yielding threshold, and m is related to the wall's apparent fluidity (reciprocal viscosity). Because P and Y are in the range 5 to 6 atmospheres, and $(P - Y)$ is roughly 0.2 atmosphere, elongation rate is initially extremely sensitive to changes in P. A small step-down in turgor (0.7 atmosphere) stops growth, and a similar rise greatly accelerates it. These initial responses are, however, soon (15 minutes) compensated by changes in Y. An apparent metabolism-dependent reaction (azide-sensitive) lowers Y; strain hardening (azide-insensitive) raises it. These two opposing processes, acting on Y, serve as a governor on $(P - Y)$, tending to maintain it at a given value despite changes in P. This ability to compensate is itself a function of turgor. Turgor step-downs are less and less well compensated, leading to lower rate, as turgor falls from 5 atmospheres to about 2 atmospheres where growth appears not to resume. This is the lowest attainable yield value, Y_1. The turgor dependency of compensation reflects a turgor requirement of the Y-lowering ("wall-softening") process. Thus the relation between steady state, r_s, and turgor is an indirect one, derived from time-dependent alterations of the cell wall. This relationship superficially resembles the instantaneously valid one in that, roughly, $r_s = (P - Y_1)m_s$. Y_1 and m_s, however, have much lower values than Y and m. The duality of the elongation rate *versus* turgor relation and the prominent role of Y in regulating rate are the major features of growth control in *Nitella*.

Plant cell growth is generally believed to be the result of a driving force, turgor pressure, acting on a yielding cell wall. Granting that both turgor and wall yielding properties will ultimately have their bases in metabolic activity, the immediate mechanism for growth could, *a priori*, be expressed as

$$r = E \cdot P \qquad (1)$$

where r is rate, E characterizes the yielding wall, and P is turgor. The attractive possibility that E is a constant and that growth rate is always proportional to P (Fig. 1, top) has to be abandoned because (*a*) the steady growth of many tissues increases with turgor only above a threshold value of turgor (1, 10, 18) and (*b*) in *Nitella* the immediate response of rate to a shift in turgor is far more than proportional to the change in turgor (7). Both objections are removed if the effective driving force is regarded as only that part of cell turgor which exceeds some yielding threshold of the wall, also measured as a pressure:

$$r = m(P - Y), \quad \text{for} \quad P > Y. \qquad (2)$$

Y is the threshold turgor for growth or apparent "yield point." The variable m is a generalized "yielding tendency" which has the units of fluidity (reciprocal viscosity). $(P - Y)$ is the effective driving force for growth. Thus if $P \leqslant Y$, $r = 0$.

The three variables, m, P, and Y, have physical units, but any or all could be under immediate metabolic control. It develops that metabolic inhibition primarily affects Y. Changes in Y account for long term growth responses.

The nature of the variables in equation 2 and their increased number pose difficulties for evaluation of the equation. Not only must P be correctly estimated in the growing cell, but now *two* parameters, Y and m, reflect properties of the cell wall. Rate would vary proportionately with m and be more than proportionately sensitive to changes in P or Y. With m and Y constant, this equation predicts a course of increase in cell length, as a function of modulated turgor, as shown in Figure 1, middle. Lockhart (9) has provided a more complex version of equation 2, where P is replaced by an expression involving the various parameters that control it. Probine and Barber (13) use a similar expression to account for *Nitella* wall yielding properties.

Equation 2 is potentially continuously solvable through all growth rate responses to turgor shift and metabolic inhibition. Three major difficulties in its solution are: (*a*) elastic effects, if large, can obscure growth rate changes; (*b*) barriers to diffusion, such as those found in tissues, may make the effect of a treatment on a tissue (hormone treatment or shift in external osmolality) gradual compared to the time course of an individual cell's response; and (*c*) turgor in the growing state may be significantly lower than at equilibrium. For the comparison of steady states (constant rates), the first two complications do not apply. Further, a turgor estimate can be made by correcting the equilibrium value (19). In *Avena* coleoptile sections, Evans (6) has compared the relation between steady rate and turgor, both with and without auxin treatment. He found that auxin lowered the apparent yield value of the wall by several atmospheres. If equation 2 is assumed to be applicable for steady rates, it can be concluded the hormone treatment increases rate by increasing the $(P - Y)$ term.

In *Nitella* it is technically feasible to evaluate periodically the terms in equation 2 by following the rate response to small step shifts in turgor. Elastic effects are minor and rapid com-

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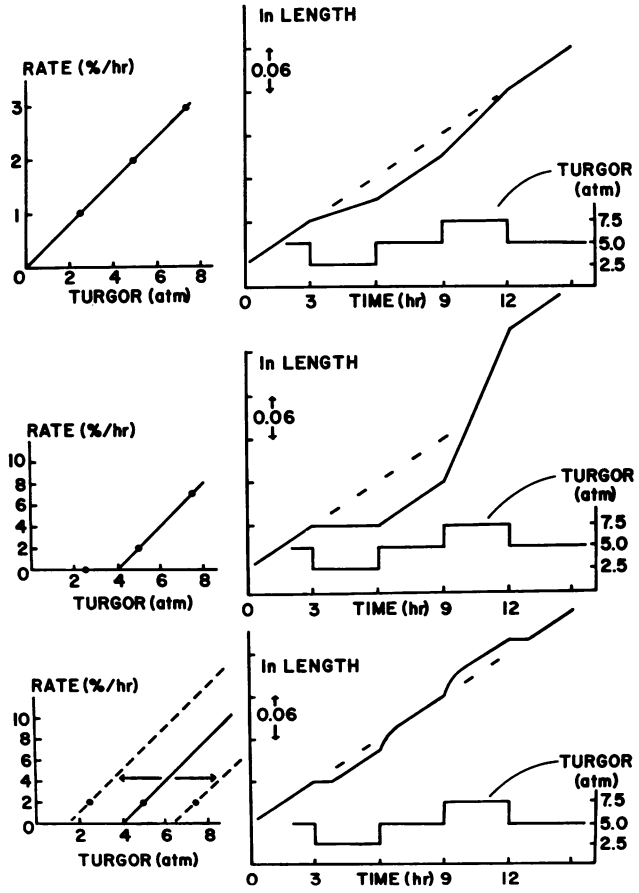

FIG. 1. Three models which relate elongation rate to turgor pressure. The graphs at left show the postulated rate *versus* turgor function; the graphs at right show the corresponding time course of length change through identical patterns of turgor shift. Top: A direct proportionality between rate and turgor, following equation 1, leads to length increase where mean rate is proportional to mean turgor. Middle: Introduction of the yield value for the wall, after equation 2, renders rate disproportionately sensitive to fractional change in turgor. Here a 50% reduction in turgor stops growth; a 50% increase almost quadruples growth rate. This sensitivity is typical for the initial rate response in *Nitella*. Note that mean rate is not proportional to mean turgor. Bottom: The same immediate relation between rate and turgor as above, but now this relation is shifted to the left when rate is slow, shifted to the right when rate is high. This shifting compensates the initial response to restore the previous rate. Note that mean rate is again proportional to mean turgor. The course of length increase shown here resembles actual growth records. See Figure 2.

pared to rate adjustments (7). An intracellular manometer technique allows the direct and continuous measurement of P. In brief, it is found that the immediate response to a turgor shift is that predicted by an equation related to equation 2 but that this response is soon compensated, by a change in Y, to bring about a return to nearly the previous rate (see Fig. 1, bottom). It is the cell's capacity to carry out this compensation that, in *Nitella*, determines the relation between turgor and steady elongation rate. This paper will analyze the hierarchy of controls governing elongation rate in this cell type.

MATERIALS AND METHODS

Principle. As before (7), turgor is measured by the capacity of a cell to compress gas trapped in the blind end of a capillary, the end of which is in the cell vacuole. A 4-mm young internode at the tip of a shoot is impaled, through its tip, with the short and open end of a fishhook-shaped capillary. Calibration of the pressure in the bubble is made by the periodic imposition of 0.5 atm of hydrostatic pressure by means of a pump connected to the vessel containing both the plant and capillary. The bubble's response is analyzed in the same film as is the course of cell growth. The cell surface is marked with anion exchange resin beads to facilitate rate measurement. Because the entire cell surface elongates, growth is measured as the relative rate, $(1/L)\ (dL/dt)$ where L is length and t is time. Use of the relative rate (*e.g.*, 1%/hr = 0.01 hr^{-1}) is convenient because this rate is independent of the absolute distance between the pair of marks used in its determination. Relative rate falls gradually during elongation, but this decline is slow compared to the frequency of turgor modulation employed.

Improvements since 1968 include modifying the rate of activity of the camera so that pictures are taken at 3-sec intervals when growth rate is expected to change rapidly, at 4-min intervals when it is not. A minute counter is imaged on the film to give a time record on each frame. The vessels containing media of varying osmotic value are given positive pressure to deliver fluid rapidly to the growth vessel. A photoconductor, with its exposure to light governed by an opaque float, controls the level of solution in the vessel. When obscured, it cuts off power to the inlet valves. Exchange of solutions takes about 30 sec.

Estimation of $(P - Y)$ and m.

Estimates of $(P - Y)$. A estimate for $(P - Y)$ can be de-

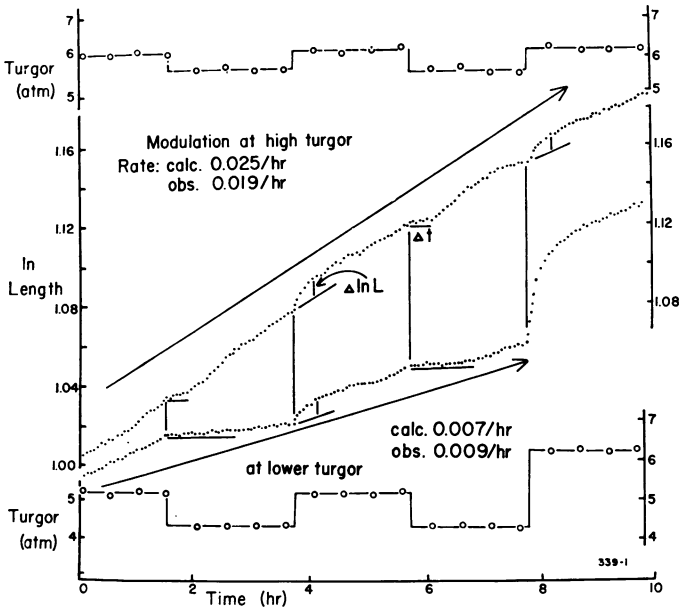

FIG. 2. Modulation of turgor, with shifts of 0.7 atm, carried out with the same cell over two turgor ranges. The time course for both turgor (open circles) and ln cell length (dots) is given. The upper pair of graphs shows that, at high turgor, a turgor step-down halts growth for about 15 min (Δt) and that a step-up leads to transitory rapid growth which gives an increment in length, $\Delta \ln L$, beyond that otherwise expected. By dividing average $\Delta \ln L$ by average Δt, one obtains a mean rate of about 2.5%/hr. The actual mean rate, shown by the long arrow, was 1.9%/hr. The lower pair of graphs shows modulation (with only one appropriately sized step-up) at a lower turgor range. Here Δt is longer and $\Delta \ln L$ appears to be about the same. The calculated rate is much lower, as is the observed rate (long arrow). The large step-up at right restores the cell to its original turgor level and rate. Generally $\Delta \ln L$ becomes disproportionately large as the size of the step-up is increased. The initial slope is also disproportionately high.

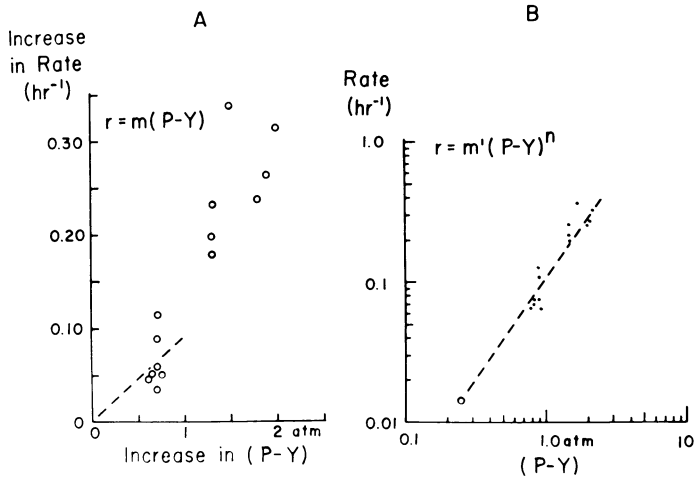

FIG. 3. Data used to estimate m and m'. The initial slope following a turgor step-up was determined by least-squares fit to the first several measurements of ln length following the turgor shift. Over these points no curvature was evident to the eye. In A, these initial slopes are plotted against the increase in $(P - Y)$ of the shift. The slope of this relation is regarded as m. For small shifts it is roughly 0.1/atm, although there is considerable scatter. In B the same data are in a double logarithmic plot. If one assumes r is proportional to $(P - Y)$ to a power greater than one, n, apparent fit is better than in A. The slope, m', is (0.15/hr)/atm^n where n is 1.4. The open circle shows normal rate (average of 18 experiments) occurring at a $(P - Y)$ of about 0.2 atm.

termined from the size of the turgor drop needed to reduce P to the level of Y, stopping growth. This stoppage has to have finite duration to be used for measurement because elastic wall shrinkage would tend to stop growth momentarily after any drop. A minor role for elasticity in the turgor response of *Nitella* is supported by the inelastic nature of the cell wall (21) where total loss of turgor brings on a shrinkage of 0.8%, the shrinkage being relatively less at the higher turgors. When shrinkage can be observed after large drops in P (Fig. 6 in Ref. 7), it is complete long before there is resumption of growth, and thus elasticity appears to be involved in only a minor way in a given stoppage. Direct measurement of shrinkage (unpublished) reveals a half-time of about 20 sec for a turgor shift of 0.7 atm. In previous work the minimal detectable turgor drop for stoppage was found to be about 0.3 atm (Fig. 4 in Ref. 7). It is quite clear from hundreds of records (*e.g.*, Figs. 2 and 4) that a drop of 0.7 atm stops growth for periods that are at least 10 to 20 min long; this is consistent with smaller drops causing a brief stop in growth. It can be concluded that $(P - Y)$, the effective driving force, is of the order of 0.3 atm, and probably less.

Estimates of m. Assuming at the start that m is a constant, it can be measured as $\Delta r/\Delta(P - Y)$. A rise in $(P - Y)$ is achieved by a sudden increase in P, brought on by a shift to a medium of reduced osmotic value; the corresponding increase in r is then measured. Evaluating m requires the estimation of initial slopes on ln (natural logarithm) length *versus* time curves, some of which are given in Figure 3A. For small rises in P the estimates are of the order of (0.1/hr)/atm. Values for m are similar for similar turgor jumps at various absolute turgor levels (Fig. 4, bottom). Large shifts in P, however, tend to give disproportionately higher values for m, as in Figure 2, lower right. For simplicity m will be regarded as approximately (0.1/hr)/atm for small shifts in P. The disproportionate effect can be expressed by changing equation 2 to $r = m'(P - Y)^n$, where n is about 1.4 and m' is (0.15/hr)/atm^n. Reasonable fit is obtained when $(P - Y)$ for mean average rate, 0.0146 hr^{-1}, is about 0.20 atm (see Fig. 3B).

The data from initial responses to turgor shift support the validity of equation 2 in that, from the range of low values calculated for $(P - Y)$ and the range of high values for m, one can select pairs of values which roughly predict typical steady rates of growth: (0.2 atm)·[(0.1/hr)/atm)] = 0.2/hr. The fact that initial responses to turgor shift are ultimately compensated (Figs. 2 and 4) indicates that at least one of the terms in equation 2 is capable of considerable change.

Stabilization of Rate: Governor-like Activity of Y. The subsequent return to near normal rate following a turgor shift is not inherent in equation 2 as written. The basis for this adjustment is sought in changes in the $(P - Y)$ term, rather than m, for the following reasons. The resumption of growth after a fall in turgor could not even in principle be accounted for by a change in m because $(P - Y)$ would remain negative (effectively zero), independently of m. The decrease in rate following the rapid growth response to a rise in turgor could, however, reflect a fall in m (increase in apparent viscosity). If this were the case, a second small rise in turgor should reveal a low value of m. Successive turgor rises of the same magnitude, however, bring on comparable initial high rates (Fig. 4). This weighs against a regulatory role for m. While some as yet unresolved variation in m may be involved, this would be small compared to the 5-fold or higher reduction in rate which compensates the initial response to a rise in turgor. The $(P - Y)$ term thus appears to be the major one involved in regulation.

The Y component of $(P - Y)$ is considered the regulatory

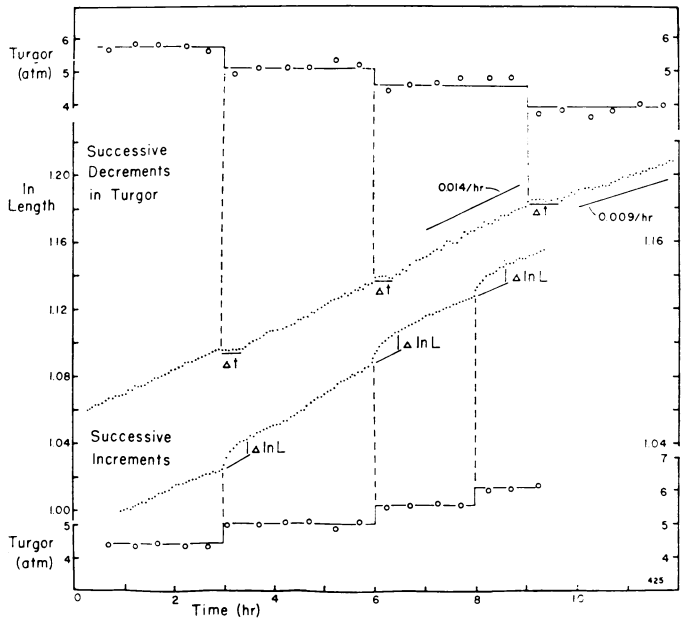

FIG. 4. Successive similar shifts in turgor applied to a single cell. Turgor (open circles) and ln length (dots) are shown as a function of time for repeated step-downs (upper graphs) and step-ups (lower graphs) of turgor. Upper pair of graphs: The first two step-downs are followed by similar lag times, Δt, before resumption of growth. This reduction in turgor from about 6 to 5 atm does not result in an obvious fall in rate. Only the third step-down increases the lag and decreases steady rate. Lower pair of graphs: Unlike the sensitivity of Δt to turgor pressure, $\Delta \ln L$ appears to be independent of turgor. Because successive step-ups show apparent identical initial slopes, it is concluded that the gradual reduction of rate after a step-up is not achieved by a reduction in m. Rate is believed to be reduced by a decrease in $(P - Y)$ brought on by a rise in Y.

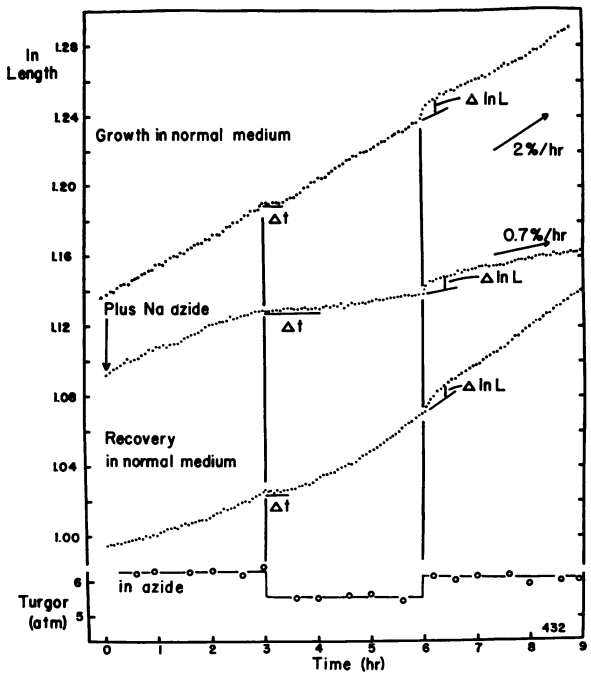

FIG. 5. Changes in rate and turgor shift transients of a single cell brought on by treatment with sodium azide (2×10^{-5} M). The top graph (dots) shows the course of increase in ln length of a cell through a turgor regime similar to the one at the bottom (open circles). Δt and Δln length are normal. In the second graph the cell is in sodium azide. Note that rate gradually falls over 2 hr to about 0.7%/hr. The step-down in turgor results in several-fold longer Δt; the step-up yields, however, a normal Δln L. The recovery of the cell (third graph) is largely over after 3 hr in normal medium, and the Δt for recovery from step-down is reduced to about the normal value. The turgor record (bottom) shows that the fall in rate and the increased duration of Δt are not related to a loss in turgor.

one because direct measurement of P reveals only small and gradual changes following a turgor shift. Cessation of growth allows apparent continued ion uptake to raise turgor slowly (7); rapid growth after a turgor rise slightly dilutes cell sap, reducing turgor. These changes are small compared to the magnitude of the turgor shift whose effects are being compensated, and so Y must be of major significance in bringing about compensation of the initial response. Thus, by elimination, a compensatory lowering of Y appears to restore $(P - Y)$ after a fall in turgor. A compensatory rise of Y, following a rise in P, is confirmed by the finding that a small drop in P will again stop growth after steady rate has been reestablished (Fig. 2). The factors influencing the value of Y thus become of special interest.

Lowering of Y, "Wall Softening." The process of yield threshold lowering, or s ("softening"), is measured by the time Δt, required for the cell to resume a steady growth rate following a fall in turgor. This assumes that the ultimate shift in Y is the same as the imposed shift in P (measured directly). If $(P - Y)$ is not the same before and after the shift, there will be an error in the estimate of the shift in Y. This error will not be large, however, when the imposed turgor shift is large compared to $(P - Y)$. Large turgor shifts, however, yield data which apply at widely different absolute turgors and hence are not desirable. A compromise is struck by using shifts of about 0.7 atm to study Y. If the steady value for $(P - Y)$ changes by 0.1 atm with a turgor drop of 0.7 atm, then the calculated shift in Y will be in error by one part is seven. Within this limitation s is measured as the estimated shift in Y divided by the time required for the shift. It has the units atm hr^{-1}. The values recorded at high turgor (above 5 atm) are about 2.4 atm/hr (Fig. 2).

As the same downward shift in P is applied to steadily growing cells at ever lower levels of absolute turgor, it is noted that resumption of steady rate ultimately takes longer (s is lower in value; see Figs. 2 and 4). Since the value of s is decreased by 2×10^{-5} M sodium azide (Fig. 5), it is a metabolic process, apparently the major one involved in the immediate mechanism of growth. Because the value also falls with reduction in turgor (it is stress-sensitive), it appears to be a chemorheological process of the type suggested by Ray (15, 17) as governing growth rate. That s may be rate-limiting is indicated by a fall in steady growth rate which parallels a fall in s (Figs. 2 and 4).

The lower limit to which Y can be reduced appears to be roughly 2 atm of turgor (7), although this limit has not been rigorously tested. This limit may coincide with the mechanical "yield point" of excised strips of *Nitella* cell walls (14). With evidence for a plateau value for s over the turgor range from 5 to 6 atm (Fig. 4), declining values from 5 to 2 atm (Figs. 2 and 4), and probably a zero value below 2 atm, s is a complicated variable. For purposes of mimicking growth responses with mathematical models, s is assumed to fall linearly to zero over the range from 5 to 2 atm (P' in Fig. 8, II). There are insufficient data to make more than a rough characterization of this curve.

Increase in Y. The increase in Y which follows a rise in P appears to be complete by the end of the "growth burst" brought on the upward shift in turgor. This is indicated by the fact that a subsequent small drop in turgor will again stop growth. No data have been obtained on the position of the yield threshold during a growth burst. Because the rise in Y and decrease in rate for a given turgor shift is about the same in the presence or absence of azide (Fig. 5), it is felt that this process is primarily physical. The physical process of strain hardening (4, 12) is appropriate to account for the phenomenon of raising Y and reducing rate after it has been increased by a turgor step-up. Stiffening by strain hardening involves the shifting, to the right along the abscissa, of a rate (ordinate) *versus* stress relation so that a given stress becomes associated with reduced strain rate. The slope is unchanged. This shifting

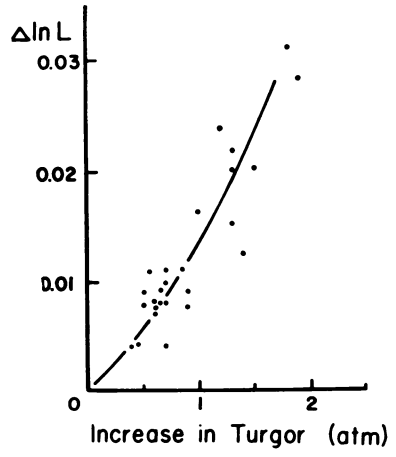

FIG. 6. A graph showing that Δln L increases more than linearly with the size of the turgor step-up. If it is assumed that simple strain hardening, h, raises Y to reduce rapid growth, the rate of raising Y would be $r \cdot h$. This should lead to a linear increase of Δln L with ΔP. The nonlinearity seen here can be accommodated in a model if it is assumed that strain hardening is less effective at higher rates. That is, $h \cdot r$ is placed by $h \cdot r^q$ where q is less than 1.0. When q equals 0.75, the appropriate nonlinearity is attained.

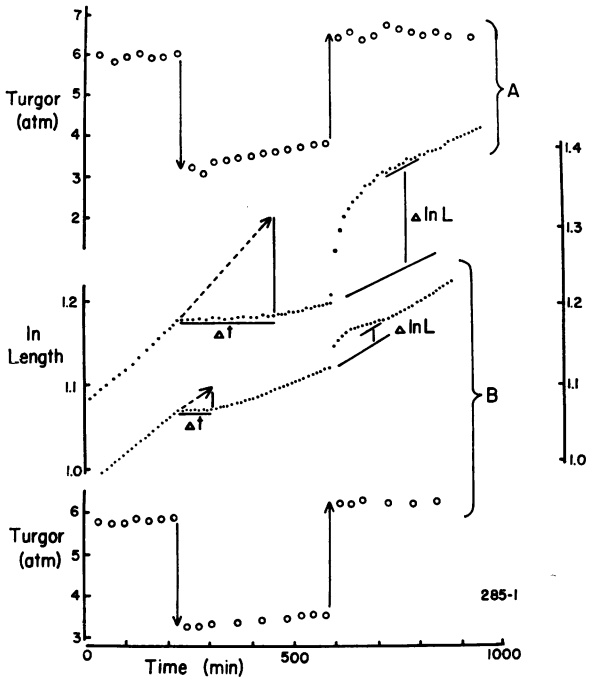

FIG. 7. Turgor (open circles) and length (dots) changes in a single cell on consecutive days. The upper pair of graphs, pertaining to the first day of growth, shows that a large drop in turgor causes a long delay (Δt) before growth is resumed. The equivalent rise leads to a great increase in ln length ($\Delta \ln L$). Placing $\Delta \ln L$ at the end of the lag period (Δt) gives a rate value (slope of the large dashed arrow) roughly equivalent to that shown by the cell at high turgor. In the lower pair of graphs showing growth on the 2nd day both Δt and $\Delta \ln L$ are reduced; their quotient, however, is about the same (slope of the small dashed arrow). The cell's mean rate is also about the same. This suggests that the quotient $\Delta \ln L/\Delta t$ ($= s/h$) determines steady rate. Measurement of either parameter alone would not correlate with elongation rate in this case. The shift in values for this cell was a response to a change in nutrient medium experienced just before the first record was taken.

carries with it a shift in the intercept with the abscissa, Y. The extent of shifting is proportional to the stretch (strain) experienced by the object. If unopposed, strain hardening would lead to cessation of strain, after a stress increase, after a certain amount of stretch had occurred. Stiffening by changing viscosity, on the other hand, involves a fall in slope of a rate *versus* stress curve. This also gives reduced rate at a given stress but does not tend to lead to a stoppage. The appropriateness of the strain hardening mode of stiffening to account for the reduction in rate which terminates a "growth burst" is based on the apparent increase in Y which accompanies the decrease in rate after the start of the growth burst. Strain hardening (h) is measured in terms of the extra length (beyond that anticipated from normal growth), $\Delta \ln L$, gained due to a single upward shift in turgor (see Figs. 2 and 4). h is the number of atm that would be required to stretch the cell by 100%. For small shifts in turgor, typically 1% increase in length is associated with a 1-atm rise in Y; hence $h = 100$ atm.

The general nature of the growth burst (roughly logarithmic decay of growth rate to an equilibrium level) is compatible with the assumption of strain hardening acting to translate an apparent fluidity curve (rate *versus* P) to higher turgors. Upon abrupt increase in P, rate is very high; hence the rate of raising Y, $r \cdot h$, is also high. As Y rises, $(P - Y)$ decreases ever more slowly, and rate and $r \cdot h$ approach the low steady state value (Fig. 10).

Examination of the growth responses to similarly sized increases in P, but at different absolute turgors, indicates that h is not a prominent function of P (Fig. 4). Comparison of the response (extra length) to turgor rises of varying magnitude, however, shows that strain hardening is less effective in the larger shifts (Figs. 2 and 6). In general, strain hardening appears to be less effective at high rates. For simple models (Fig. 1, bottom) this can be ignored and the strain hardening function is $h \cdot r$. For more detailed models one can incorporate disproportionality by assuming that the rate of raising Y is $h' \cdot r^q$ where q is 0.75.

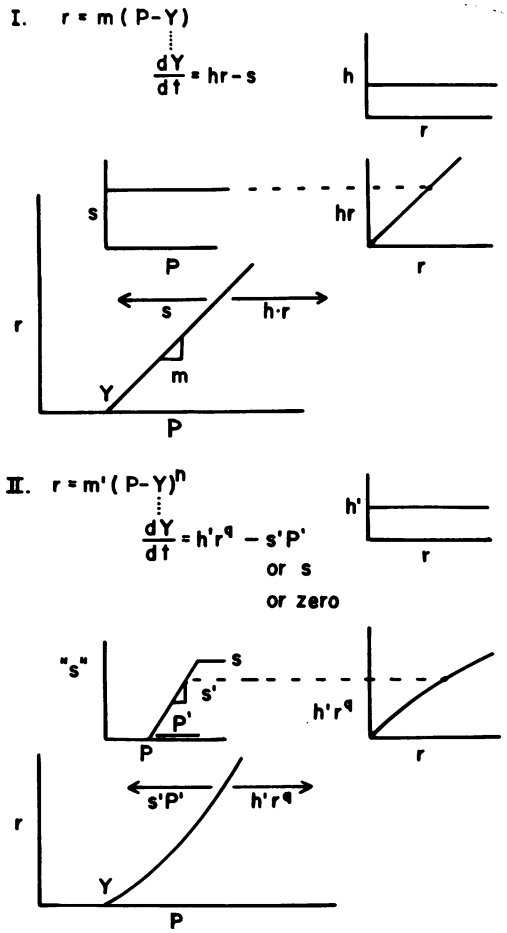

FIG. 8. Graphical representations of simple (I) and complex (II) models for growth regulation in *Nitella*. I: The growth equation assumes that rate is simply proportional to $(P - Y)$. Y in turn is raised by $h \cdot r$ and lowered by a constant value of s. Thus s is constant with P and $h \cdot r$ is a linear function of r. Steady rate is achieved by a balance between s and $h \cdot r$ as shown by the dashed horizontal line. Transient rate effects are predicted by the time course of the transposition of the rate *versus* turgor relation. This model is applicable only over the turgor range 5 to 6 atm for small shifts in turgor (about 0.7 atm). II: A more complex expression is used for the rate *versus* turgor relation. As shown in the lowest graph, this is concave upward. The factors that move this curve along the turgor axis are also more complex. It is moved to the left by "s," which now is a function of turgor in that over the restricted range of turgor, P', it rises linearly to a plateau. The expression that moves the curve to the right is also more involved in that the rate of movement ($h' \cdot r^q$) does not increase linearly with rate but is concave downward. Nonetheless, steady rate is attained when the value for "s" equals $h' \cdot r^q$, as shown by the horizontal dashed line. The additional complexity allows the model to fit growth responses at a variety of turgor pressures and after various sizes of turgor shift.

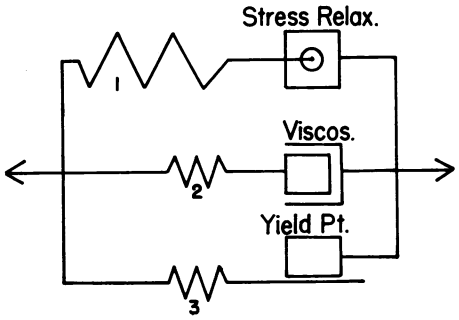

FIG. 9. A rheological model to explain the fall of apparent yield threshold (Y) in terms of a redistribution of stress, following a fall in stress, rather than a change in a yield point (St. Venant) body. If spring 1 is soft and greatly extended during elongation, it can bear most of the stress on the system. When stress is reduced (turgor step-down), the system will stop yielding because the yield threshold is not exceeded. Since, however, spring 1 is in series with a stress relaxation device ("wire-spinning machine") a redistribution of stress will occur after the stoppage. Stress on spring 1 will be continually reduced, and so the other elements will carry a larger and larger proportion. This redistribution could lead to resumption of extension if the threshold stress for the yield point body is exceeded. In *Nitella* spring 1 may be soft, allowing effects of such a redistribution to be readily detected. In *Avena*, spring 1 may be stiff, and so little or no effective redistribution would occur. The viscous properties of the wall (m) would be a function of the dash-pot in the middle line, and the small elastic effects in *Nitella* would relate to properties of springs 2 and 3. This model possibility was pointed out by Dr. P. M. Ray (Stanford University, Stanford, Calif.).

Stabilization of Rate by Interaction of s and h. In view of the stability of m and P, steady growth rate at constant turgor can be expected only when Y is constant. Since in simple models s lowers Y, and $h \cdot r$ raises it,

$$\frac{dY}{dt} = hr - s \qquad (3)$$

There is no change in Y ($dY/dt = 0$) when $r = s/h$. Thus, if s lowers Y at 2 atm/hr, h must raise it 2 atm in an hour. If h is 100, or 1 atm/1% stretch, then the cell would elongate at 2%/hr. This interaction is seen graphically when the increase in ln length during a growth burst ($\Delta \ln L$) is placed at the late end of the lag period, Δt, required for the resumption of growth after the equivalent turgor drop (Fig. 7). This forms two sides of a triangle. It is noted that the third side roughly approximates the course of normal growth between turgor shifts. The approximation becomes less crude as the amplitude of turgor shifts diminishes (Figs. 2 and 4). This indicates that s and h operate during steady growth and, in combination, govern equilibrium growth rate. When this relationship is incorporated into equation 2, expressions for the course of cell length (L) through stepshifts in turgor ($P_1 - P_0$) may be derived. For steady growth ($P_1 = P_0$) and turgor step-ups ($P_1 > P_0$):

$$\ln L = \ln L_0 + \frac{s}{h} \cdot t + \frac{(P_1 - P_0)}{h}(1 - e^{-mht}) \qquad (4)$$

For step-downs ($P_1 < P_0$) insufficient to stop growth [$(P_1 - P_0) < (P_0 - Y_0)$], equation 4 applies. Y_0 is the value of Y, after equation 2, at the start of a turgor shift. For step-downs which stop growth, [$P_0 - P_1) \geq (P_0 - Y_0)$], there is no growth for the period $(Y_0 - P_1)/s$; then equation 4 applies (5). This equation would predict the behavior shown in Figure 1, bottom, and embodies the graphical model in Figure 8 (I). This gives a fair simulation of actual responses for small turgor shifts at high turgor, especially when it is recognized that, aside from boundary terms (initial length, initial Y and P) it requires that only three biological variables be specified (m, s, and h). In order to provide a mathematical model which will simulate growth responses over a wide range of turgor and over varying degrees of turgor shift, as already noted, it is necessary to introduce additional constants and more elaborate expressions. The result does not yield an integral in closed form because of the fractional exponents involved. The relationships summarized below can be integrated, by computer approximation, to give artificial growth curves which simulate

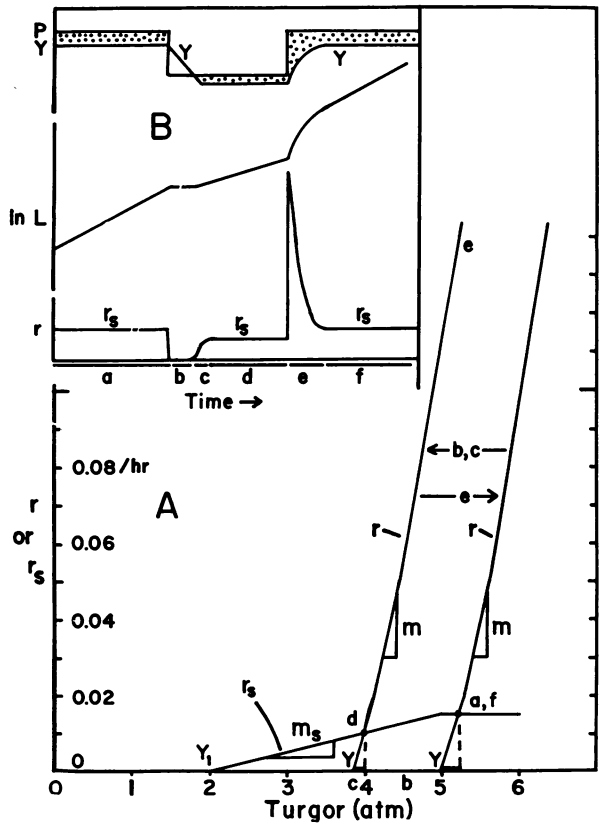

FIG. 10. The dual relationship between rate and turgor pressure, approximately to scale. Both relations are evident in a cell's response to temporary fall in turgor pressure. A: The relation between steady rate, r_s, and turgor is given by the gently rising curve, with slope m_s, at bottom. The lowest yield point (and turgor) compatible with growth is shown as Y_1. The instantaneous relation between rate and turgor is given in the steep curves (with slope m or variable slope related to $(P - Y)^n$). A steadily growing cell at 5.2 atm turgor (a) will have a yield point (Y) at about 5 atm. If turgor falls to 4 atm, growth will cease because Y is no longer exceeded. $(P - Y)$ is shown by the thick bar on the abscissa. The instantaneous curve shifts to the left (b). As Y reaches 4 atm, elongation will resume and accelerate, c, until a new but lower steady rate is reached (d). Abrupt return to 5.2 atm turgor will initially give an extremely high rate, over 0.1 hr^{-1}, which declines as the instantaneous curve moves to the right (e) and stabilizes again at the previous rate (f). Thus a cell does not change steady rates by simply shifting rate as indicated by the r_s curve but rather shows transient effects involving the immediate relation with turgor. The time course of the rate responses described in A is given graphically in B where turgor is P, ln L is ln length, and r is rate. The stippled area shows $(P - Y)$, the effective driving force of growth, when it has positive values. When it is negative, growth ceases. Note that the decline in Y is linear because it is result of continuous metabolic action; the rise in Y is nonlinear since it is brought about by strain hardening which raises Y more rapidly when elongation is rapid.

actual data (5). This indicates that the general nature of the functions and the approximations used to provide the necessary constants from the available data are adequate. The relationships are:

$$r = m'(P - Y)^n \quad (5)$$

where

$$\frac{dY}{dt} = h' \cdot r^q - s \quad (6)$$

Appropriate constants are: $m' = (0.15/\text{hr})/\text{atm}^n$; $n = 1.4$; $h' = 57$ atm; and $q = 0.75$. $s = 2.4$ atm hr^{-1} at 5 to 6 atm turgor and increases from 0 to 2.4 atm over the turgor range from 2 to 5 atm. Use of these values simulates growth as seen in Figures 2 and 4.

DISCUSSION

The major conclusion of this paper is that one can describe several interrelated levels of control of elongation rate in *Nitella*. (*a*) Immediate or primary control is embodied in the equation $r = (P - Y)m$ (equation 2) and minor modifications of it. It accounts for the immediate rate response to turgor shift. (*b*) Restoration of steady rate following minor turgor perturbation is found to be based on the cell's capacity to adjust Y so as to re-establish approximately the previous value of $(P - Y)$. Adjustment of Y involves the interaction of a metabolic Y-lowering process, s, operating roughly at 2 atm/hr at high turgor, and apparent strain hardening, h, which typically has a value of the order of 1 atm/1% stretch. A constant Y (and rate) will result if a cell elongates at 2%/hr. This governor-like action of s and h is the second level of control. (*c*) The question of what steady rate is maintained involves the next level of regulation. Cells grow slowly or not at all at 2 atm turgor, increase their steady rate until a turgor of about 5 atm is reached, and then maintain this rate at least through a turgor of 6 atm (see Fig. 2). This turgor sensitivity of steady rate, r_s, can be traced to the turgor sensitivity of s. (*d*) Higher levels of variation were noted but not studied carefully. Over long periods of time several of the "constant" parameters change in value. Osmoregulation may occur to change turgor (7), and the value of s often appears to rise slowly with time, even at essentially constant turgor (compare rate at two successive treatments at 4 atm turgor in Fig. 2, bottom). A parallel shift in both s and h, involving roughly a 5-fold change in each, is seen in Figure 7. This discussion will center on the first three levels of control: how they interact to give two rate *versus*-turgor relations, and their possible generality.

Two Rate versus Turgor Relations. Because both the immediate control of rate and the higher level of control involving stabilization of steady rate are turgor-dependent, there is one relation between instantaneous rate and turgor, another one between steady rate and turgor. Both are shown in Figure 10. They have a superficial similarity in that both involve a threshold value for elongation and, above that point, an increase in rate as a function of turgor. Quantitatively, however, the relations are quite different. As shown in Figure 10, a normally growing cell with 6 atm of turgor is operating just above the immediate yield threshold, Y. It will stop growth upon a turgor step-down of only 0.3 atm. Its capacity to ultimately adjust to a drop in turgor, given a period of time for s to act unopposed by h, is such that it could later resume growth, albeit at a lower rate, after a step-down as large as 2.5 to 3 atm (7). Thus there is a apparent lowest attainable yield value, Y_1, about equal to the lowest turgor compatible with elongation. For comparison purposes it becomes important to know which rate *versus* turgor relation is under study. In the absence of special efforts to follow transient effects, it is likely that most information pertains to steady rates.

Similar Controls in Other Systems?

Immediate Control of Rate. Measurements of m, P, Y, comparable to those presented here, appear not to have been made on other systems. Since a threshold turgor for elongation is widespread, it is assumed that a relation similar to equation 2 is general. Several studies accurately followed changes in elongation rate of tissues subjected to stimulatory or inhibitory treatment. It is clear from the work of Ray (15) and Ray and Ruesink (19) that in *Avena* coleoptile sections rate is closely dependent on metabolism. Rate response to change in temperature, metabolic inhibition, or auxin stimulation was complete within several minutes. In some cases rate change appeared to be limited by the time course of penetration of the agent into the tissue. Such data tend to rule out a large value for $(P - Y)$, with m a constant physical fluidity. In such an arrangement growth would cease only when $(P - Y)$ reached zero, presumably by a rise in Y. With metabolic inhibition of extension, one would expect considerable elongation during the period of decline of $(P - Y)$. Since such "coasting" is not found, one may assume that Y is close to P or that it is m which is under metabolic control.

Stabilization of Rate. A two-factor control of rate (as against a single "rate-limiting reaction"), similar to the governor-like control proposed here, has been suggested by Cleland (2, 4). Under imposed mechanical stress (Instron technique), killed *Avena* coleoptile sections which had been grown in auxin showed a lower value for strain hardening than did control sections. Viscosity appeared to be a minor element in the extension of the tissue (see also Ref. 10). It was concluded that (*a*) growth was based on a succession of yielding events wherein metabolism would lower Y a finite amount below P and then extension (strain hardening) would raise it, and (*b*) that auxin could, in part, accelerate growth by reducing strain hardening.

Lockhart (unpublished) has found, with living but plasmolyzed mung bean tissue, that repeated application of a weight gave larger extensions, per application, as the interval between applications increased. This would be interpreted as follows: During the unloaded period a metabolic reaction (s) lowered Y more and more. Extension, combined with strain hardening, then raised it to halt deformation. This would imply rate control by s/h. An alternate explanation could involve metabolic action on m during stoppage of extension.

A mechanochemical model for control of elongation in plants contrasts with the current view for growth of bacteria (22) where the "softening" action is lytic and the "stiffening" event is the catalyzed covalent cross-linking of cell wall polymers. Many studies have proposed an analogous softening role for polysaccharidases in plant growth (17). Since, however, s in *Nitella* is closely linked to metabolism, and the activity of lytic enzymes generally is not, it seems more likely that the softening action involves synthesis and/or incorporation of material into the wall (see "Discussion" in Ref. 17). It is possible that, within the wall, a site of stressed bonding between polymers could be changed to a site of relaxed bonding by the formation of new (unstressed) bonds with new polymer that had entered the site. Ray (16) has shown that new cell wall material enters the wall interior during growth in *Avena*. This effect is enhanced by auxin.

Ray (personal communication) has pointed out that the apparent Y-lowering process seen in *Nitella*, s, need not be the result of a change in a yield threshold in the sense of that of a St. Venant body (solid friction element) in rheological terminology, but could result from the redistribution of

stresses within a complex rheological system. His model is shown in Figure 9, where an invariant yield threshold element is in parallel with a soft spring (1). During elongation this spring is greatly extended and bears much of the stress. Upon a fall in stress, growth will stop if the amount of stress on the yield body not exceed its threshold. With time, however, the stress borne by the soft spring will diminish owing to the continued activity of a stress relaxation device ("wire spinning machine") with which it is in series. The fraction of the now reduced stress carried by the yield element will increase. When its yield threshold is exceeded, elongation will resume. In this way "yield point lowering" may reflect stress-relaxation.

Change from One Stabilized Rate to Another. In *Nitella* steady rate is a function of turgor, and this can be accounted for by the turgor sensitivity of s. The most widely studied alteration of steady rate is the shift to more rapid elongation in many higher plant tissues brought on by auxin. If it is assumed that rate is governed by s/h, and the evidence for this is summarized above, then stimulation could involve one or both terms. As noted, the observed decrease in h detected in killed tissue (2, 4) could account for some of the rate increase. Because, however, the time required for h to fall was long compared to the attainment of the full growth response, it was concluded that other factors, presumably s, were involved in the early response. A failure of changes in mechanically measured strain hardening to correlate well with growth rate (11, 20) also appears to put more of a burden on s.

A pressure sensitivity of s, as noted in *Nitella*, can be surmised from a later study (3) where turgor pressure was found to enhance the auxin-induced lowering of h (measured mechanically). This enhancement occurred mainly over the turgor range 0 to about 6 atm, a range over which growth is minimal. Above 6 atm of turgor, elongation rate increases with turgor but h does not. By elimination it would appear that s has larger values at higher turgor pressures.

Evans (6) showed that auxin treatment lowered Y_1 in *Avena* coleoptile sections from 7.8 to 3.4 atm, greatly increasing $(P - Y_1)$. If Y_1 were equivalent to Y, this change would account for most of the stimulation of growth. For the reasons given above, however, Y is probably much closer to P than is Y_1. If so, the apparent auxin effects on s and h would involve a relatively small $(P - Y)$ and possibly resemble the promoting action of increased turgor on growth rate in *Nitella*.

If an s/h control of rate is general, there arises the possibility that growth regulators, and their interactions, may involve both terms. The same may be said for the unknown controls which govern the fall-off of rate, with position, that determines the shape of tip-growing cells (8). There is no obvious way to distinguish the alternative sites of control other than by relating a regulator's effects on transient behavior to its action on steady rate.

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